Composition and Antimicrobial Activity of Juniperus Communis L. Physiological Metabolites from Serbia

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***Abstract*-Juniperus communis L. (Cupresaceae) is widely distributed in the forest region of Serbia. In addition to ecological significance as a pioneer species in forest regeneration, it is also of important medicinal plant. Its fruit produces a valuable essential oil. The composition of the essential oil from Juniperus communis was analyzed by GC-MS, and microbiological assays were carried out. Samples were collected in different localities of Zlatar Mountain, (west Serbia). Of the total isolated 42 compounds, identified is 32. The major compounds in the essential oils were α-pinene, sabinene, p-cymene, l-limonene, and α-terpinolene. Also, both qualitative and quantitative differences between different parts of the plant were observed. The essential oils and their major compounds were tested against Agrobacterium tumefaciens, Bacillus subtilis, Esherichia coli and Pseudomonas flurescens. The obtained results indicate to a significant inhibitory effect essential oils on growth many pathogenic microorganisms.**

Keywords-Juniperus communis; essential oil; antimicrobial activity

I. INTRODUCTION

Among the numerous plant functions, one of the most important role is that they produce secondary metabolites that have a significant antimicrobial activity. The essential oils, which exist as secondary metabolites of aromatic plants, are very important (M o r r is *et al.*, 1979; J e n s e n *et al.*, 1987; A d e b a j o *et al.*, 1989; A n g i o n i *et al.*, 2003). The antimicrobial activity of the plants is significant for the existence of dynamical ecological balance which is the basic condition for the life on the Earth. As a result of this activity, very old and complex plant communities exist at present time (M a t o v i ć *et al*., 1995).

Juniperus communis is a species with a great ecological amplitude. In Serbia, it occurs from Hungarian oak-Turkey oak forests and forests with Eastern hornbeam at foothills, beech-fir forests and spruce forests, to devastated pastures over very diverse geological substrates and soil types. Ecological diversity of sites where juniper occurs, as well as its geological age tertiary resulted in great variability, i.e. adaptation to the specific conditions (ecotypes, chemotypes, varieties, etc.).

The oil of *J. communis* has been used for centauries as diuretic, based on its terpinan-4-ol contant. The plant is also used in folk medicine as carnitative, antiseptic, and as remedy for indigestion It is well documented for its medicinal value for diarrhea, abdominal pain, tumors piles, bronchitis and indigestion in traditional system of medicine (S a t i *et al*., 2010). In herbal medicine, juniper oil has been used as a carnitative, diuretic and as a steam inhalant in management tuberculosis and diabetes. It has also been used in arthritis as well as antioxidant (T a r a s c o v a *et al.*, 1995). Juniper berries have long been used as favoring agents in foods and alcoholic beverages.. Some of antimicrobial properties of the essential oil have also been reported. Juniperus are widely used in compounding spices, and oil is used in perfumes, pharmaceutical and cosmetic products.

Changes in the content and composition of an essential oil from Juniper can be caused by environmental factors, such as soil or climate in which the plants are grown, and by different harvesting methods or distillation techniques (M i l l e r, 1951). As a rule, essential oil yield is lower in the regions situated north from ex Yugoslavia. The average contents of essential oil in juniper fruits were reported to be cca 2.45%, 1-1.5%, 1-1.2%, 0.8-1.7% in France, Germany, Chezka and Russia, respectively, and 0.2-0.5% in Serbia (J o v a n o v i ć, 1992).

In mountain area of western Serbia, there is poor information on the composition and antimicrobial activity of essential oil from genus Juniper (M a t o v i ć *et al.*, 1995; 1998). Therefore, the present study was carried out to examine the antimicrobial activity of different plant parts of *Juniperus communis*, that occur of the Zlatar Mt (west Serbia), against of some pathogenic microorganisms.

II. MATERIAL AND METHODS

A. Plant Material and Isolation Procedure

The aerial parts of *Juniperus communis* L. have been collected at Zlatar Mt (west Serbia) from 2003 to 2007. They were removed manually. The voucher specimens were deposited at the herbarium and berries were air-dried at room temperature. They were stored in paper bags at ambient temperature, protected from light and well air-conditioned in order to prevent fermentation.

Dried aerial parts (200gr) of plants were subjected to the hydro-distillation by a standard procedure (Clevenger apparatus) according to the method recommended by the European Pharmacopoeia (2001) to produce oil. The oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis. The samples used in this analysis were: milled fruits; milled remains of the fruits after

the alcohol extraction; alcohol extract from the fruits; milled remains of the fruits after the water extraction; water extract of the fruits; milled remains of the fruits after the distillation of the essential oils; essential oil from the fruits; milled leaves.

B. Fractionation

Batch distillation was performed in a pilot plant. A column of 2 m height was charged with Normag packing. It was determined experimentally that the column had 36 theoretical stages. Fractionation on the essential oil was conducted at absolute pressures of 26 and 66 mbar with a reflux ratio of 2-5. Fractions of 10 cm^3 were collected during the fractional distillation. The fraction of essential oil with supercritical carbon dioxide was performed in an Autoclave Engineers SCE in two experiments under different conditional (175 bar and 40°C using supercritical carbon dioxide with 5 wt% of methanol as co-solvent and at 90 bar and 75°C with pure carbon dioxide).

C. GC/MS Analysis

Samples dissolved in n-hexane were subjected to gas chromatographic analysis on a Varian 3400 gas chromatograph equipped with an FID (HP 5890 Series II, HP 5971 MSD, electron impact mode 70) (A d a m s, 1995). A fused silica DB-5 capillary column, 25 m x 0.32 mm internal diameter and 0.25 µm film thickness was used. The purged spitless mode of sampling was implemented. The column temperature was maintained at 50°C for 5 min and programmed to increase as follows: at 3°C/min to 240°C and holding at 250°C for 5 min. The flow rate of the carrier gas (nitrogen) through column was 2 ml min^{-1}. The injector temperature was 250°C at the linear temperature program from 40-280°C and detector temperature was 300°C. The temperature of the transfer line was 280°C.

The Mass Spectrometer model SSQ 700 equipped with library software Wiley 138 and NBS 75 was used. Compounds were identified by matching their mass spectra with those recorded in the MS library and further confirmed by injecting the authentic samples of different available compounds with the volatile oil and by comparing the mass spectra with those of reference compounds.

D. Antimicrobial Activity

Antimicrobial activity of the investigated metabolites of *Juniperus communis* was determined by the disc-diffusion method and by measuring the inhibition zone. The bacterial strains used in this study were: *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Esherichia coli* and *Pseudomonas flurescens*. The bacterial strains were obtained from the Faculty of Science, University of Niš.

Agar diffusion method was carried out according to Collins and Lyne (1985). Nutrient agar (NA) was used for cultivation of bacteria. In this method, pasteurized paper discs (0,5 mm in diameter) were impregnated with 100 ml of each oil and applied on the surface of agar plates freshly seeded with standard inoculums of yang cultures, 24 h old bacteria. The plates of test organisms were than incubated at 20°C for 48 h. At the end of the incubation period the inhibition zones were measured (the experiment were designed, the results are averages of triplicates).

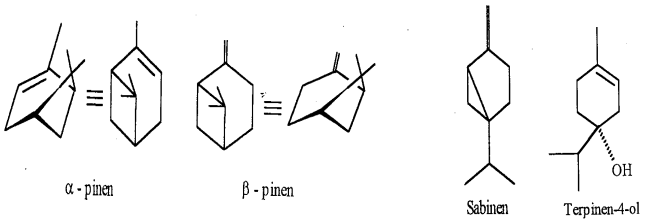


Figure1. The effect of the Juniperus communis metabolite to the Agrobacterium tumefaciens population density

III. RESULTS AND DISCUSSION

The quantity of essential oils of *Juniperus communis* fruits from eight localities at Zlatar Mt. (Serbia) was from 3,25% to 2,13%. In the essential oil from *Juniperus communis* was registered 42 components. From these 42 components, 32 was identified and quantified. It was found that α-pinene was the major constituent of essential oils (30,763%). Sabinene was also found in considerable amounts (19,37%). Except those, in larger quantities identified following compounds: ß-mycrene (16,42%), epi-seskquifelandrene (6,38%), l-limonene (4,904%), γ-elemene (3,28%), ß-elemene (2,06%) α-terpinolene (1,318%), *p*-cymene (0,23%) etc.(Table 1).

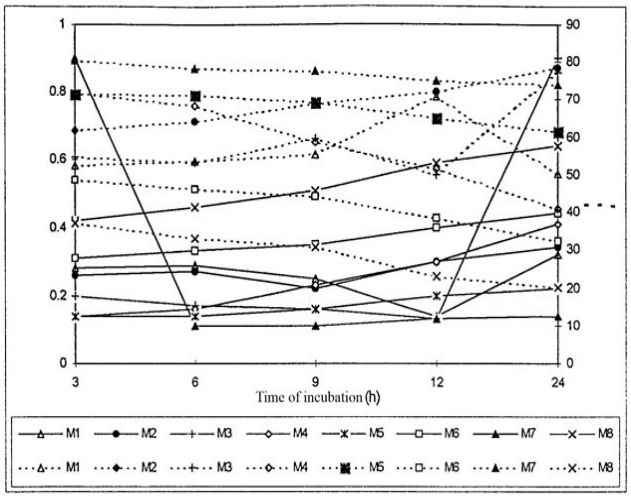


Figure2. The effect of the Juniperus communis metabolites to the Bacillus subtilis population density

On the growth of population and metabolic activity of *Bacillus suptilis* milled residues fruit berries after distillation of essential oils and milled needles of juniper did not affect, while the other of metabolites inhibited the growth of this bacteria (Fig. 2).

Among the tested of microorganisms, *Escherichia coli* was the least sensitive to metabolites of *Juniperus communis*. Only, milled berries remains after extraction of alcohol inhibited the growth of this population, while in presence of other metabolites *E. coli* freely developed (Fig. 3).

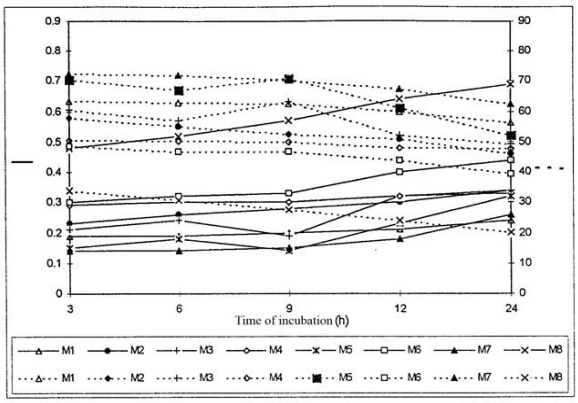


Figure3. The effect of the Juniperus communis metabolites to the Esherichia coli population density

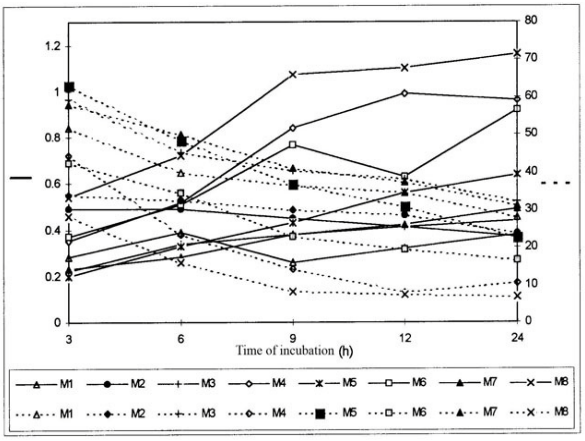
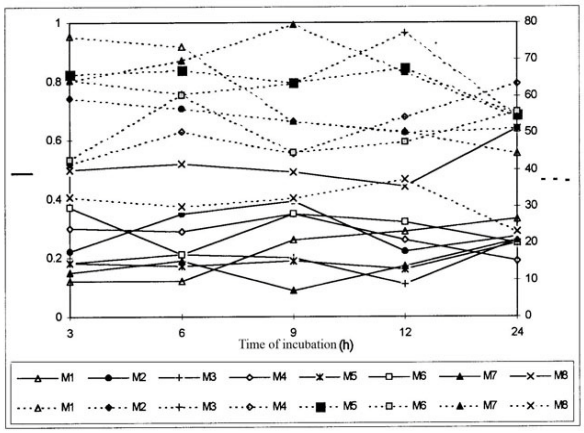


Figure4. The effect of the Juniperus communis metabolites to the Pseudomonas fluorescens population density



Legend for Figures 1 –4: M1-milled fruits; M2-milled remains of the fruits after the alcohol extraction; M3- alcoholic extract from the fruits; M4-milled remains of the fruits after the water extraction; M5-water extract of the fruits; M6--milled remains of the fruits after the distillation of the essential oils; M7-essential oil from the fruits; M8-milled leaves of Juniperus communis

TABLE1. GS-MS ANALYSIS OF THE ESSENTIAL OILS OF JUNIPERUS COMMUNIS

	Rt min.	**Compounds**	**CI***	**%**	**% Rel.**
1	14.120	n.i.**		0.091	0.296
2	15.339	n.i.**		0.113	0.367
3	21.506	α-tujen (91)	938	1.465	4.762
4	21.974	α -pinen (93)	942	30.763	100.00
5	22.646	kamfen (97)	954	0.196	0.638
6	23.622	sabinene (91)	976	19.372	62.972
7	23.918	β-pinen (94)	981	2.154	7.002
8	24.163	β-micren (91)	986	16.427	53.399
9	24.865	n.i.**		0.154	0.502
10	25.424	δ-3-karen (90)		0.081	0.262
11	25.597	α-terpinen (97)		0.404	1.314
12	25.739	p-cimen (91)	1020	0.226	0.734
13	26.187	l-limonen (96)	1030	4.904	15.94
14	27.396	γ-terpinen (96)	1057	0.787	2.557
15	27.753	trans-sabinenhidrat (59)		0.178	0.579
16	28.758	α-terpinolen (96)		1.318	4.286
17	28.880	linalol (38)	1092	0.073	0.237
18	32.537	terpinen-4-ol (97)	1175	1.087	3.535
19	32.964	α-terpineol (83)	1185	0.146	0.474
20	34.811	n.i.**		0.234	0.762
21	36.578	n.i.**		0.073	0.238
22	36.752	bornilacetat (99)		0.289	0.939
23	38.759	citronelilacetat (91)	1335	0.171	0.555
24	39.052	neoizotujil alcohol (72)		0.136	0.443
25	39.305	n.i.**		0.081	0.263
26	39.710	nerilacetat (59)	1345	0.117	0.380
27	38.812	α-kubenen (98)	1344	0.609	1.979
28	40.962	α-kopanen (97)	1369	0.645	2.098
29	41.298	(-)-β-elemen (95)		2.063	6.706
30	41.916	epizonaren (94)		0.475	1.543
31	42.494	n.i.**		0.154	0.500
32	42.677	γ-elemen (98)	1425	3.278	10.655
33	42.923	β-farnezen (90)		1.002	3.259
34	43.361	junipen (45)		0.067	0.219
35	43.799	α-humulen (98)	1437	1.421	4.618
36	44.280	n.i.**		0.167	0.544
37	44.649	epibicikloseskvifelandren (93)		6.376	20.725
38	45.008	α-muurolen (92)		0.264	0.858
39	45.570	γ-kadinen (98)	1510	0.345	1.121
40	45.683	δ-kadinen (99)	1524	1.282	4.168
41	46.449	n.i.**		0.121	0.394
42	47.655	n.i.**		0.690	2.243

The negative effect of metabolites from juniper on *Pseudomonas fluorescens* was confirmed in water extract of the fruit (Fig. 4).

The previous data shows that the antimicrobial products of *Juniperus communis* have an inhibitory effect which is limited by its chemical content, concentration and by the taxonomical

properties of the microorganisms (R a m i c and M u r k o, 1983; M a t o v i ć et al., 1996; 1997; P e p e l j n j a k *et al*., 2005; S a w a i *et al*., 2007)

Our research has shown that physiological metabolites of juniper in shape the essential oil have great significance for maintaining the dynamic equilibrium of the environment. Because a major component of α-pinene, oil possesses a characteristic odor and exerts antimicrobial action in the ionized air. In the oil is also present cyclic monoterpene alcohol terpinen-4-oil. Its diuretic effect is manifested in nature, and therefore the essential oil of juniper is used as a diuretic agent in modern phytotherapy.

This study established that the milled whole fruits, milled remains of the fruits after the alcohol extraction, milled remains of the fruits after hydro-distillation, water extract of the fruits, milled remains of the fruits after the distillation of the essential oils and essential oil of *Juniperus communis* are inhibiting the growth of *Agrobacterium tumefaciens, Bacillus subtilis and Pseudomonas fluorescens*. An alcohol extract and the milled leaves of *Juniperus communis* are inhibiting the growth of *Agrobacterium tumefaciens* and *Bacillus subtilis* (Figures 1 – 4).

The growth of *Agrobacterium tumifaciens* was different in the presence of various metabolites. Milled juniper needles stimulated the growth of these bacteria, probably as a result of the presence and content of growth factors that were found in metabolites of needles. Other metabolites were inhibited the growth of population in greater or lesser extent (Fig.1).

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